

CHROMOSOME PAIRING IN LARIX SPECIES

HALLY JOLIVETTE SAX

With plate 51 and one text figure

THE CONIFERS show a remarkable uniformity in chromosome number. With few exceptions all the genera have twelve pairs of chromosomes (Tischler 1927). No polyploid species have been reported, and the meiotic divisions seem to be very regular. Chromosome behavior in species hybrids has never been described in the Gymnosperms.

This study was undertaken to determine whether or not there is any cytological basis for the uniformity in chromosome numbers in the Conifers, and to analyze the chromosome behavior in a species hybrid. The meiotic chromosomes have been examined in *Pinus*, *Tsuga*, *Taxus*, *Picea*, *Pseudolarix*, *Cedrus*, and *Larix*. The meiotic chromosomes are similar in all the above genera. The present analysis will be confined to two species of *Larix* and an F_1 hybrid. A more detailed analysis of chiasma formation and chromatid relationships at meiosis will be published later.

Larix eurolepis Henry is a hybrid between *L. K mpferi* Sarg. and *L. decidua* Mill. This hybrid is intermediate in appearance between the parental species, but possesses considerable hybrid vigor and matures its cones earlier than either parent. An account of the origin and characteristics of this hybrid has been recently published by Anderson (1931).

Larix K mpferi is a native of Japan, while *L. decidua* is indigenous to northern and central Europe (Rehder 1927). This distribution of the two species would indicate that the parental types had been separated for long periods of time.

The material for the present paper was taken from specimens of these Larches growing in the Arboretum. The divisions in the pollen mother cells occurred during very cold weather. The time of division in the hybrid was between that of the two parents. During the last week in February the pollen mother cells were found dividing in *L. K mpferi*. *L. eurolepis* produced dividing pollen mother cells throughout the first week in March. The divisions in *L. decidua* occurred the following week. Other specimens of *L. decidua*, from a different part of the Arboretum and from a different slope, were a little more advanced in this respect. Material was obtained over a period of several days in each case.

Permanent smears of the pollen mother cells were made. A number of fixatives were used. Darlington's modification of Flemming's fixative proved to be the best for *Larix*. The crystal violet-iodine stain gave the best results for work on the chromosomes.

Within a given genus chiasma frequency in the meiotic chromosomes is evidently a good index of chromosome affinities and provides an accurate method for measuring the degree of chromosome pairing in species and generic hybrids. Aase (1930) noted the greater frequency of univalents and open (single chiasma) types of bivalents in *Triticum* \times *Aegilops* hybrids than in the parents. Although Aase did not use the term "chiasma" in her description of chromosome pairing, the different types of bivalents were observed and discussed. Darlington (1931b) and Hollingshead (1932) have corroborated Aase's observations in *Triticum* hybrids, and they have discussed the problem from the standpoint of chiasma frequency.

THE MEIOTIC CHROMOSOMES.

The twelve bivalents from one nucleus of *Larix decidua* are shown in figure 1. These were drawn from a side view of the metaphase figure. Each chromosome was drawn separately so that details of pairing might be observed. The chromosomes of *L. eurolepis*, the hybrid, are shown in figure 2, and those of *L. Kämpferi*, in figure 3.

The nodes are undoubtedly chiasmata, and the cross formed by the exchange of partners among the four chromatids can be observed in many chromosomes. Most of the chiasmata are approximately median or subterminal in respect to the spindle fiber attachment and the distal ends of the chromosomes. In many cases the chiasmata are terminalized with difficulty, and the chromosomes are stretched into thin strands between the fiber attachments and the chiasmata. (Figure 2b and c.) Consecutive internodes are oriented in planes more or less at right angles to each other. There is a wide separation of free arms at the distal side of the chiasmata as well as between the chiasmata. In the smear preparations no spindle fibers were observed, although, with the same fixative, paraffin sections show clear spindle fibers.

Pairing of bivalent chromosomes in *Larix* and other Conifers seems to be effected only by chiasmata,—a condition which Darlington (1931a) believes to be true for all genera. The prevalence of interstitial chiasmata at diakinesis and early metaphase indicates that there is little terminalization of chiasmata during the prophase stages of meiosis.

CHIASMA FREQUENCY IN *LARIX* SPECIES AND F₁ HYBRID

Chiasma frequency can easily be observed in *Larix* at diakinesis and metaphase of the meiotic divisions. Counts were made in pollen mother cells where all twelve bivalents could be clearly observed. The data are summarized in Table I.

TABLE I
Chiasma frequency in *Larix* species and F₂ hybrids

	Number of cells counted	Number of Chiasmata						Total number of chromosomes	Average number of chiasmata
		0	1	2	3	4	5		
<i>Larix decidua</i>	37		19 4%	279 63%	110 25%	36 8%	0	444	2.36 ± .02
<i>Larix eurolepis</i>	51	4 7%	49 8%	294 48%	198 32%	66 10.7%	1 .001%	612	2.45 ± .02
<i>Larix Kämpferi</i>	46		88 16%	223 40%	139 25%	98 18%	4 .007%	552	2.47 ± .03

In *Larix decidua* the chiasma frequency ranges from one to four per bivalent with an average of 2.36. In *L. Kämpferi* the range is from one to five with an average of 2.47 chiasmata per bivalent. No univalents or precocious divisions were found in these species. In the F₁ hybrid (*L. eurolepis*) the chiasma frequency is from zero to five, and the average per bivalent is 2.45. The hybrid is intermediate between the two parents, although the chiasma frequency is nearer that found in *L. Kämpferi*. The differences in mean chiasma frequency are of doubtful significance, although these differences between the parents and between *L. decidua* and the hybrid are about three times the probable error. Univalents or precocious divisions of one bivalent were found in about eight per cent of the pollen mother cells of the hybrid. In practically all cases observed, these single chromosomes were passing to opposite poles, so that a normal distribution of chromosomes would be expected.

The distribution of chiasma frequency is shown graphically in Text figure 1. The curve of chiasma frequency in the hybrid is intermediate between those of the parental species, but the differences in the curves are of doubtful significance. The shape of the curves is similar to the condition found in *Vicia* by Mæda (1930),

which Haldane (1931) attributes to interference in chiasma formation.



FIG. 1. CHIASMA FREQUENCY IN LARIX.

In view of the great regularity in the chromosome number, as well as the similarity in chiasma frequency between the parents and the hybrid, very little pollen sterility was expected. Counts of good and bad pollen in the parents and hybrid show greater sterility in the hybrid than in either parent. The counts and percentages of poor pollen are recorded in Table II.

TABLE II
Pollen sterility in Larix species and F₁ hybrid

	good pollen	poor pollen	percentage of poor pollen
Larix decidua	614	14	2.2%
Larix eurolepis (L. Kämpferi × L. decidua)	866	85	9.2%
Larix Kämpferi	1030	29	2.7%

DISCUSSION

The uniformity in chromosome numbers for most genera of the Gymnosperms, and the absence of recorded cases of polyploidy, can perhaps be attributed to the type and frequency of chiasma formation at meiosis. With an average of about 2.4 interstitial chiasmata per bivalent, any autopolyploids produced would be expected to form closely paired tetravalents. The segregation of homologous chromosomes in such polyploids would probably be too irregular to produce a high degree of fertility, and the polyploid would have small chance of survival. Very few polyploid species occur in nature where the chromosomes pair as quadrivalents at meiosis. The only known case where a high percentage of quadrivalents is found in fertile species is in the tetraploid *Tradescantias* (Anderson and Diehl 1932). In these species the spindle fiber attachment point is approximately median, and the chiasmata are largely terminal in both diploids and tetraploids. The chromosome morphology and types of chiasmata formed might permit rather free movements of the homologous members of a quadrivalent, so that regular chromosome distribution and a high degree of fertility might be expected. With a high frequency of interstitial chiasmata, regular assortment and fertility would not be expected in autopolyploids. The absence of polyploid species of Conifers may also be dependent on other factors, in addition to the type and frequency of chiasma formation.

If chiasma frequency is a good index of chromosome homology, as has been shown by several writers, the chromosome constitutions of *L. decidua* and *L. Kæmpferi* appear to be very similar. The average chiasma frequency is approximately the same in the parental species and the F_1 hybrid. There is, however, a tendency toward weak pairing between two of the homologous chromosomes in the hybrid. In about eight per cent of the figures examined, two homologous chromosomes were not paired at early metaphase, although these two homologous chromosomes seem to pass to opposite poles. Apparently these two chromosomes are not completely homologous, but the average chiasma frequency found in the hybrid is not lower than that found in the parents.

About nine per cent of the pollen in the F_1 hybrid is morphologically imperfect as compared with two or three per cent found in the parental species. The increased pollen sterility of the hybrid can not be due to irregularities in chromosome distribution at meiosis.

SUMMARY

A detailed analysis was made of chromosome pairing in *Larix Kæmpferi*, *Larix decidua*, and a hybrid between these two species (*Larix eurolepis*). The chromosome number and the type of chiasma formation is similar in the three species and in the remainder of Conifers studied. The comparatively high number of chiasmata, together with the prevalence of the interstitial chiasmata formed here, may account largely for the great uniformity in chromosome number and general stability in the group.

The chiasma frequency was remarkably similar in all three species. The average chiasmata frequency per bivalent was 2.36 for *L. decidua*, 2.47 for *L. Kæmpferi*, and 2.45 for *L. eurolepis*.

Although there were a few cases where there was weaker pairing in the homologues in one bivalent of the hybrid, chromosome distribution appeared to be regular, and the average chiasma frequency was as high as that in the parents. The pollen sterility was somewhat greater in the hybrid than in the parents. This greater pollen sterility in the hybrid can not be attributed to irregular chromosome distribution or to difference in chiasma frequency.

LITERATURE CITED

- AASE, HANNAH (1930). Cytology of Triticum, Secale and Aegilops hybrids with reference to phylogeny. (Research studies of the State College of Washington, 2: 4-60.)
- ANDERSON, EDGAR (1931). Hybrid trees. (Arnold Arb. Bull. Pop. Inform. 5: 65-68.)
- ANDERSON, EDGAR & D. G. DIEHL (1932). Contributions to the Tradescantia problem. (Jour. Arnold Arb. 13: 213-231.)
- DARLINGTON, C. D. (1931a). Meiosis. (Biol. Rev. 6: 221-264.)
- . (1931b). The analysis of chromosome pairing in Triticum hybrids. (Cytologia, 3: 21-25.)
- HALDANE, J. B. S. (1931). The cytological basis of genetical interference. (Cytologia, 3: 55-65.)
- HOLLINGSHEAD, LILLIAN (1932). The occurrence of unpaired chromosomes in hybrids between varieties of Triticum vulgare. (Cytologia, 3: 119-141.)
- MÆDA, T. (1930). On the configurations of gemini in the pollen mother cells of Vicia Faba L. (Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 5: 124-137.)
- REHDER, ALFRED (1927). Manual of cultivated trees and shrubs. The Macmillan Co.
- TISCHLER, G. (1927). Pflanzliche Chromosomen-Zahlen. (Tabulæ Biologicae. Sonderabdruck aus Bd. IV.)